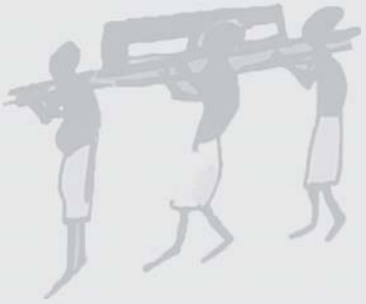

Chapter 5C

Linezolid safety and tolerability in multidrug-resistant tuberculosis patients: a retrospective observational study

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Submitted

ABSTRACT

Objectives

Linezolid, known for its toxicity, is a promising drug for the treatment of multidrug-resistant tuberculosis (MDR-TB). Dose reduction has been studied attempting to limit toxicity, but concerns exist that dose reduction could result in inadequate linezolid exposure.

Methods

We aimed to investigate linezolid safety and tolerability in relation to linezolid exposure in a retrospective study at two tuberculosis centers in the Netherlands and Italy.

Results

A total of 58 MDR-TB patients was included. No correlation was observed between microscopy or culture conversion and the area under the time concentration curve / minimal inhibitory concentration ratio. Patients that experienced peripheral neuropathy had received a higher median cumulative dose or received linezolid for a longer median period of time, compared to patients without peripheral neuropathy.

Conclusions

Treatment regimens containing linezolid were effective and well tolerated. Peripheral neuropathy seemed to be mediated by cumulative linezolid dose and number of days of exposure to linezolid.

INTRODUCTION

The prevalence and incidence of multidrug-resistant tuberculosis (MDR-TB) cases, caused by *M. tuberculosis* strains resistant to at least rifampicin and isoniazid, is increasing in high tuberculosis burden countries and is expected to keep rising in the next three years (1). The World Health Organization (WHO) recommends to prescribe an MDR-TB therapeutic regimen consisting of at least four *in vitro* active anti-tuberculosis drugs (2-6).

Linezolid is a promising anti-tuberculosis drug for the treatment of MDR-TB (7, 8) and may be added to anti-tuberculosis regimens requiring a Group 5 drug. Recently, two systematic reviews and meta-analyses pointed out its excellent efficacy (9, 10). However, linezolid toxicity may outweigh its potential benefits. Indeed, adverse events were notified in almost 60% of the treated cases, with a high incidence of severe events such as anemia, peripheral neuropathy, optic neuritis, and thrombocytopenia. Dose reduction has been studied in an attempt to limit the toxicity. Decreased doses were associated with significantly lowered toxicity (11). Nevertheless, concerns might exist about the loss of efficacy or the emergence of acquired resistance, since dose reduction might result in inadequate linezolid exposure.

Therapeutic drug monitoring (TDM) has increasingly been recognized as an asset in the field of tuberculosis treatment several years ago (12-14). TDM may be adjunct in assessing individual linezolid exposure, especially since linezolid pharmacokinetics show a large inter-individual variability (15) and important drug-drug interactions have been observed (16, 17). Defining predictors for inter- or intra-patient variability might help in identifying patients at risk for deviating exposure. Preliminary data show that area under the time concentration curve (AUC)/minimal inhibitory concentration (MIC) ratio may be the pharmacokinetic / pharmacodynamic (PK/PD) target for *Mycobacterium tuberculosis* in order to adjust dosages based on TDM results (18). Furthermore, the evidence on correlation between reduced linezolid exposure and lowered toxicity is limited. Therefore, we aimed to retrospectively investigate linezolid safety and tolerability in relation to linezolid exposure.

METHODS

Study setting and participants

A retrospective study was conducted at two tuberculosis reference hospitals: the Tuberculosis Center Beatrixoord (University Medical Center Groningen, Haren, The Netherlands) and

the Tuberculosis Reference Center for MDR-TB and HIV-TB E. Morelli Hospital (Sondalo, Italy). We selected multi- and extensively-drug resistant tuberculosis (XDR-TB) patients that received linezolid as a part of their treatment regimen from 2010 – 2012 in Sondalo and from 2007 – 2012 in Haren (4). Patients had been diagnosed by means of standard microbiological culture tests. Patients younger than 18 years, patients lacking data due to recent admission to either reference hospital, and patients of whom no TDM data was available were excluded from the study.

Drug susceptibility testing and sample analysis

Data from drug susceptibility testing (DST) and pharmacokinetic analyses was collected retrospectively. DST was performed by the National Mycobacteria Reference Laboratory (National Institute for Public Health and the Environment, Bilthoven, the Netherlands) for patients in Haren or by the WHO Supranational Reference Laboratory (Milan, Italy) for patients in Sondalo. Exact MICs were determined for linezolid (19).

In Haren, linezolid samples were analyzed using a liquid chromatography tandem mass-spectrometric method (20). Samples obtained from patients in Sondalo were analyzed at the Luigi Sacco hospital (Milan, Italy) using a high performance liquid chromatography method (21).

Data collection

Anonymous retrospective data were retrieved by two researchers. The Institutional Review Board of the University Medical Center Groningen waived the requirement for research subjects to give informed consent (METc 2013/492). Date of admission, gender, country of birth, WHO Region of birth were collected. We calculated body mass index (kg/m^2) at time of admission. In case of diabetes mellitus type II co-morbidity and HIV co-infection, clinical information including treatment was recorded. Besides these variables, detailed information on the DST and the location of infection (extra-pulmonary and/or pulmonary, including radiographic findings) based on findings of the attending physician were noted. Treatment information consisted of treatment outcome, duration of treatment in months, and cumulative composition of the administered treatment regimen.

Pharmacokinetic data consisted of $AUC_{0-12/24}$, the highest and the lowest measured concentration (C_{max}, and C_{min}, respectively). AUCs were calculated using trapezoidal, non-

compartmental, no lag-time calculations. Date of sampling, number of days after starting linezolid, linezolid dose at time of sampling, co-medication during linezolid sampling were noted in regard to linezolid pharmacokinetics. In Haren, dose reduction was considered if the calculated AUC_{0-24h}/MIC ratio exceeds 100 (12, 15, 18). After dose reduction, linezolid TDM was repeated at steady state. If full pharmacokinetic curves were available for different doses, the curve of the dose that was administered during the longest period of time was used for the analysis.

Treatment outcome was assessed besides interim status through collecting data on days to sputum smear (SS) or culture conversion, *i.e.* time in number of days between the first positive and first of two consecutive negative samples. To assess safety and tolerability, adverse events, *e.g.* leucopenia, peripheral neuropathy, and optic neuritis, were collected from the hospital records or laboratory data using local reference values. Patients in Haren, as opposed to patients in Sondalo, received prophylactic erythropoietine. Information on hemoglobin (Hb in mmol/L) was recorded at respectively day 0, 30, 60, 90, 120, and 150 after the first dose of linezolid, regardless of treatment duration of linezolid.

Statistical analysis

Statistical evaluation was performed using SPSS 20 (SPSS, Chicago, IL, USA). Baseline data of patients from both hospitals were compared using Student's t test was performed for parameters that were normally distributed. Levene's test for equality of variances was used to determine whether equal variances may or may not be assumed. Independent sample Mann-Whitney U tests were used for parameters that were not normally distributed. Frequency distributions were compared using Pearson Chi-square test for categorical parameters; in case the necessary assumptions were not met, Fisher's Exact test was used.

RESULTS

In both centers, a combined number of 58 MDR/XDR-TB patients that had received linezolid and underwent TDM were included. Figure 1 depicts a schematic summary of the selection process of the patients at both centers. From 54 enrolled patients in Sondalo, 36 were excluded: 6 due to insufficient data due to recent admission and 27 due to absence of pharmacokinetic data of linezolid. Demographic and clinical characteristics of the individuals included in the study are described in Table 1. Due to the different geographical

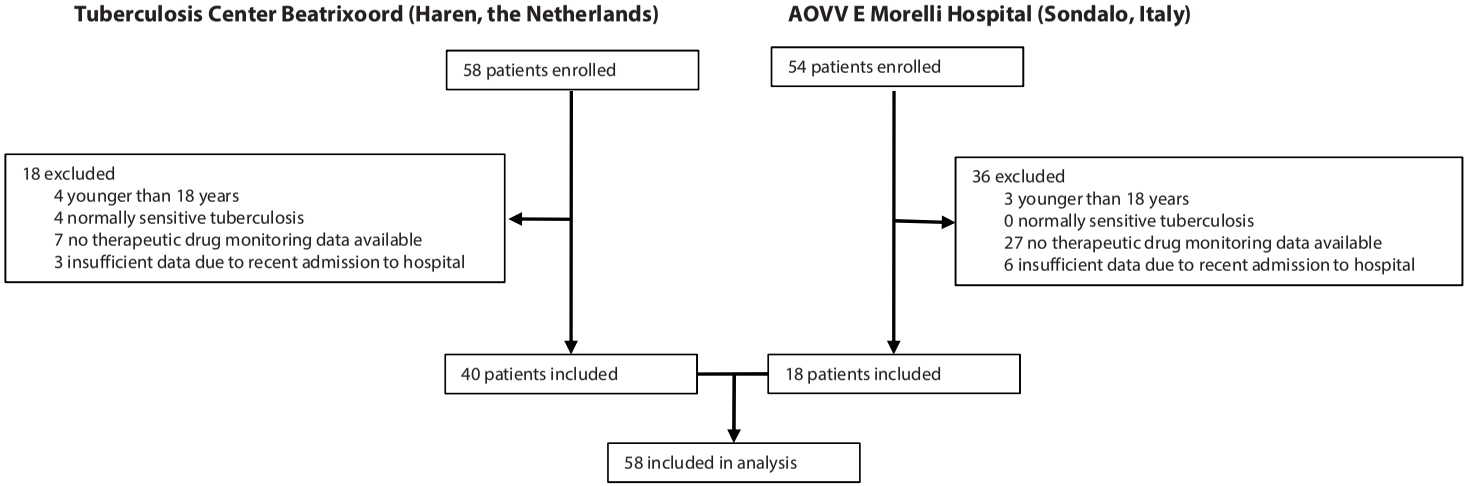

Figure 1 Schematic summary of patient selection.

Table 1 Socio-demographic and clinical characteristics in 58 patients with multidrug-resistant tuberculosis treated at Sondalo (Italy) and Haren (the Netherlands)

	All patients (n=58)	Sondalo (n=18)	Haren (n=40)	p-value
Age (years; median, IQR)	30.0 (25.0 – 37.3)	35.0 (29.8 – 40.3)	29.5 (23.3 –33.5)	0.075
HIV status				
Negative	52 (90%)	17 (94%)	35 (88%)	
Positive	4 (7%)	1 (6%)	3 (8%)	
Missing	2 (3%)	0 (0%)	2 (4%)	
Exposure to ART	3	1	2	
Sex				0.337
Female	28 (48%)	7 (39%)	21 (52%)	
Male	30 (52%)	11 (61%)	19 (48%)	
WHO Region of origin				
Africa	5 (9%)	1 (6%)	4 (10%)	
South East Asia	4 (7%)	1 (6%)	3 (8%)	
Eastern Mediterranean	14 (24%)	2 (11%)	12 (30%)	
Americas	3 (5%)	0 (0%)	3 (8%)	
Europe	27 (47%)	14 (78%)	13 (32%)	
Western Pacific	5 (9%)	0 (0%)	5 (12%)	
Weight (kg; median, IQR)	62.2 (55.6 – 69.9)	64.0 (58.0 – 70.0)	60.9 (55.5 – 69.4)	0.550
BMI (kg/m^2; median, IQR)	21.2 (19.3 – 23.7)	21.4 (18.4 – 23.2)	21.0 (19.5 – 23.8)	0.455
Radiology findings				
Cavitary lesions	16 (28%)	7 (39%)	9 (22%)	
Bilateral pulmonary involvement with cavitary lesions	7 (12%)	4 (22%)	3 (8%)	
Bilateral pulmonary involvement without cavitary lesions	8 (14%)	4 (22%)	4 (10%)	
Noncavitary nonbilateral pulmonary	19 (33%)	3 (17%)	16(40%)	
Extrapulmonary tuberculosis	8 (14%)	0 (0%)	8 (20%)	

IQR: interquartile range; HIV: human immunodeficiency virus; ART: antiretroviral therapy; WHO: World Health Organization; BMI: body mass index; *Fisher's Exact Test.
The t-test for equality of means between Sondalo and Haren was used for normally distributed parameters, i.e. age, weight and BMI. Normality was confirmed using Shapiro-Wilk test. Frequency distribution of sex between patients in Sondalo and Haren is calculated using the Pearson Chi-square test.

setting and different policy concerning migrants of both countries and, thus, the influx of migrants, the WHO Region of origin of patients differed for both hospitals. In Sondalo, most patients were from Europe (n=14, 77.8%), whereas in Haren, most patients were from Europe (n=13, 32.5%) and the Eastern Mediterranean (n=12, 30.0%). In one case from Sondalo, radiology revealed extra-pulmonary tuberculosis. The referral center in Sondalo mostly received complicated infectious cases and does not admit extra-pulmonary TB cases for a long period of time.

DST revealed susceptibility to a median (interquartile range, IQR) number of 6.0 (4.0 – 8.0) drugs and resistance to 8.0 (7.0 – 10.0) drugs. Treatment regimens of 58 MDR/XDR-TB patients are displayed in Table 2. The median (IQR) number of different active drugs

Table 2 Treatment regimen composition in 58 patients with multidrug-resistant tuberculosis treated at Sondalo (Italy) and Haren (the Netherlands)

	All patients (n=58)	Sondalo (n=18)	Haren (n=40)	p-value
Drug susceptibility of Mtb (Number of drugs; median, IQR)				
Susceptible	6.0 (4.0 – 8.0)	7.0 (5.8 – 8.3)	5.0 (4.0 – 7.8)	0.071
Resistant	8.0 (7.0 –10.0)	8.5 (6.8 – 9.3)	8.0 (7.0 – 10.0)	0.534
Drugs used:				
Number of anti-TB drugs (median, IQR)	6.0 (5.0 – 7.0)	7.0 (6.0 – 7.3)	5.0 (4.0 – 6.0)	0.004
Group 1:				
Ethambutol	26 (45%)	5 (28%)	21 (52%)	0.080
Pyrazinamide	14 (24%)	3 (17%)	11 (28%)	0.513*
Rifabutin	4 (7%)	0 (0%)	4 (10%)	0.300*
Group 2:				
Aminoglycosides	52 (90%)	16 (89%)	36 (90%)	1.000*
Kanamycin	*7 (12%)*	*0 (0%)*	*7 (18%)*	*0.087**
Amikacin	*45 (78%)*	*16 (89%)*	*29 (73%)*	*0.307**
Capreomycin	*1 (2%)*	*0 (0%)*	*1 (3%)*	*1.000**
Group 3:				
Moxifloxacin	55 (95%)	17 (94%)	38 (95%)	1.000*
Levofloxacin	1 (2%)	0 (0%)	1 (3%)	1.000*
Group 4:				
Ethionamide †	18 (31%)	4 (22%)	14 (35%)	0.330
Cycloserine	19 (33%)	17 (94%)	2 (5%)	<0.001
PAS	10 (17%)	9 (50%)	1 (3%)	<0.001*
Group 5:				
Clofazimine	30 (52%)	8 (44%)	22 (55%)	0.457
Amoxicillin-clavulanic acid	17 (29%)	15 (83%)	2 (5%)	<0.001
Thioacetazon	1 (2%)	0 (0%)	1 (3%)	1.000*
Clarithromycin	12 (21%)	0 (0%)	12 (30%)	0.011*
Other drugs				
Meropenem	13 (22%)	13 (72%)	0 (0%)	<0.001*
Cotrimoxazole	8 (14%)	0 (0%)	8 (20%)	0.048*

Mtb: Mycobacterium tuberculosis; IQR: interquartile range; TB: tuberculosis; † consists of data of both protionamide and ethionamide; *Fisher's Exact Test.

To test whether the drug susceptibility and number of drugs used at any time during treatment was the same in both hospitals the Independent Sample Mann Whitney U test was used. Frequency distribution of number drugs between Sondalo and Haren are calculated using the Pearson Chi-square test. When assumptions for the Pearson Chi-square test were not met, 2-sided Fisher's Exact Test was used.

administered at any one time point during treatment was 6.0 (5.0 – 7.0). Patients in Sondalo received more different drugs compared to Haren (p=0.004), with a median (IQR) of 7.0 (6.0 – 7.3) drugs in Sondalo and 5.0 (4.0 – 6.0) drugs in Haren. DST revealed a median (IQR) MIC for linezolid of 0.5 (0.25 – 0.5) mg/L.

There were some notable differences in treatment regimens between the two centers: in Sondalo, patients received an aggressive treatment regimen including moxifloxacin, amikacin, linezolid, meropenem and/or amoxicillin/clavulanic acid if patients were very ill or DST revealed lack of active oral options. Two patients in Sondalo received bedaquiline as a part of a compassionate use program (22). In Sondalo, meropenem was administered to 13 (72.2%) patients, in contrast to none of the patients in Haren (p<0.001). Furthermore, WHO group 4 drug cycloserine was administered to almost all patients in Sondalo (94%), but was rarely (5%) administered to patients in Haren (p<0.001) due to differences in the availability of tuberculosis medication (23).

In Haren, identification of MDR-TB isolates by rapid molecular tests was followed by empirical treatment including kanamycin IV in doses adjusted using TDM, resulting in relatively low dosage of around 7 mg/kg. Usually, after 6 months treatment was switched to oral clofazimine; moxifloxacin (24); linezolid (12); and clarithromycin (12, 25). Cotrimoxazole was administered to 8 (20.0%) of the included patients in Haren based on DST (26, 27), compared to none (0.0%) in Sondalo (p=0.048).

Efficacy

Of the 58 included patients, 29 (50.0%) patients were considered cured and 4 (6.9%) patients completed their treatment; 25 (43.1%) patients were still on treatment. All but one patient (98.3%), including those still on treatment, were culture and smear-microscopy negative at the time of data collection. Of the individuals with positive sputum smear and cultures at the beginning of the tuberculosis treatment, median (IQR) time to conversion was 47 (21 – 68) days for sputum smear and 47 (22 – 61) days for culture. There was no significant difference between time to microscopy or culture conversion between the two participating hospitals (p=0.062 and p=0.781, respectively). There was a low correlation between AUC/MIC ratio and parameters predictive of treatment outcome, *i.e.* days to sputum smear-microscopy (r^2_{linear} = 0.364) or to culture conversion (r^2_{linear} = 0.169). The same lack of correlation applies for linezolid C_{min}, C_{max}, dose in mg/kg/day and days to culture or microscopy conversion with no linear r^2_{linear} higher than 0.138. When cases are categorized based AUC/MIC ratio using 100 as a cutoff,

which is a commonly used target based on other microorganisms, the distribution of days to microscopy (p=0.984) or culture conversion (p=0.241) was the same across both groups.

Adverse events

Linezolid and erythropoietin were co-administered for a median (IQR) of 88 (62 – 142) days. Baseline hemoglobin levels (median; IQR) of patients that did (8.0; 7.1 – 8.6 mmol/L) or did not receive erythropoietin at the start of linezolid therapy (8.3; 7.1 – 9.5 mmol/L) were similar (p=0.452) (Table 3). Furthermore, at all other time points up to 90 days there was no difference between hemoglobin of patients that did or did not receive erythropoietin, nor was there a difference in hemoglobin over time per patient between the two groups (Table 3). There were too few patients with data on days 120 – 180 available to allow for adequate interpretation of these data. None of the patients in Haren received blood transfusions.

Table 4 describes the cumulative dose, days of exposure to linezolid, AUC_{0-24h}, and C_{min} of linezolid for patients with and without linezolid-related adverse events. Peripheral neuropathy was observed in 11 (19%) of the patients. The distribution of linezolid cumulative dosage in mg/kg (p=0.041) and days of exposure to linezolid (p=0.003) differed across patient groups with and without peripheral neuropathy. The cumulative linezolid dose was significantly higher in patients with peripheral neuropathy. They had a median (IQR) of 1,829 (1,414 – 2,255) mg/kg versus those without neuropathy that had a median (IQR) of 1,221 (742 – 1,994) mg/kg (p=0.041). A similar observation was made for the number of linezolid treatment days: median (IQR) 159 (120 – 196) days in patients with and 97

Table 3 Hemoglobin (mmol/L) at day 0, day 30, day 60, and day 90 after the first administration of linezolid

	Erythropoietin				
	Yes	n	No	n	p-value
Day 0	8.0 (7.1 – 8.6)	30	8.3 (7.1 – 9.5)	27	0.452
Day 30	7.8 (7.0 – 8.4)	29	7.9 (6.8 – 8.7)	27	0.863
Day 60	7.9 (7.1 – 8.9)	23	7.8 (6.9 – 8.8)	22	0.946
Day 90	7.5 (6.6 – 8.9)	15	7.7 (6.6 – 8.3)	15	0.967

Data presented as median (interquartile range) of hemoglobin (mmol/L). Erythropoietin was administered in a dose of 2000 IE two times a week. Data of day 120, day 150 and day 180 are not presented due to limited number of data. Mann Whitney U test (Wilcoxon rank sum) was used to test whether the distribution of hemoglobin concentrations were the same with or without erythropoietin at every time point.

Table 4 Cumulative dose, days of exposure to linezolid, AUC_{0-24h}, and C_{min} of linezolid for patients with and without linezolid-related adverse events

	N (%)	Linezolid cumulative dose (mg/kg)	p-value	Total linezolid exposure (days)	p-value	Linezolid AUC_{0-24h} (mg*h/L)	p-value	Linezolid C_{min} (mg/L)	p-value
Anemia									
Yes	9 (16%)	918 (550 – 1964)	0.480	97 (52 – 159)	0.768	158 (117 – 218)	0.299	3.4 (2.5 – 5.5)	0.169
No	30 (52%)	1211 (855 – 1830)		97 (66 – 150)		128 (98 – 170)		2.5 (1.5 – 4.0)	
Leukopenia									
Yes	5 (9%)	1898 (1056 – 2668)	0.194	150 (102 – 352)	0.065	158 (134 – 178)	0.314	3.8 (3.0 – 4.4)	0.207
No	40 (69%)	1046 (739 – 1813)		86 (62 – 150)		122 (89 – 171)		2.6 (1.4 – 4.2)	
Peripheral neuropathy									
Yes	11 (19%)	1829 (1414 – 2255)	0.041	159 (120 – 196)	0.003	149 (104 – 185)	0.261	3.2 (1.6 – 4.1)	0.477
No	25 (43%)	1164 (755 – 1922)		97 (66 – 147)		107 (91 – 156)		2.0 (1.4 – 3.8)	
Optical neuropathy									
Yes	1 (2%)	997	0.735	89	0.766	100	0.612	2.5	0.98
No	48 (83%)	1306 (861 – 1985)		105 (67 – 169)		127 (96 – 169)		2.6 (1.5 – 4.0)	

N: number of patients (percentage of the total number of patients included in the study, i.e. 58); Anemia: <8.7 mmol/L (♂), <7.5 mmol/L (♀); Leukopenia: $<4*10^9$/L (♂,♀).
Linezolid AUC_{0-24h}: area under the time linezolid concentration curve from 0-24 hours; Linezolid C_{min}: lowest measured linezolid concentration.
Mann Whitney U test was used to test whether the distribution of parameters was the same across groups with or without anemia, leucopenia, peripheral neuropathy, and optical neuropathy respectively.

(66 – 147) days in patients without peripheral neuropathy (p=0.003). Nine percent (5/45; 8 missing data) had leucopenia: no difference was identified in cumulative linezolid dose nor in days of exposure to linezolid (p=0.194 and 0.065, respectively). One patient had optical neuropathy.

The distribution of AUC_{0-24h} was the same for patients with and without anemia (p=0.299), leucopenia (p=0.314), optical neuritis (p=0.612), and peripheral neuropathy (p=0.261). The distribution of C_{min} of linezolid was the same across the categories anemia, leucopenia, optical neuritis, and peripheral neuropathy. Only two of 48 patients (2/48; 10 missing data) had diabetes type 2, rendering combined analysis of adverse events and diabetes impossible.

Pharmacokinetic parameters

Patients had a mean (standard deviation, SD) C_{min} of 3.1 (2.2) mg/L, a C_{max} of 9.4 (3.1) mg/L, and an AUC_{0-12h} of 70.1 (31.9) mg*h/L with all different dosages combined. Pharmacokinetic parameters for the different dose categories are displayed in Table 5. Due to the small number of patients receiving 400 mg, 900 mg, or 1,200 mg linezolid respectively, comparison of pharmacokinetic parameters of these groups was not possible.

There was no correlation between linezolid dose in mg/kg and AUC_{0-12h} with or without excluding P-glycoprotein modulators (r^2_{linear} were 0.012 and 0.001, respectively). Patients that concomitantly received P-gp inhibitors did not display statistically significant different means for any of the pharmacokinetic parameters. Furthermore, ideal body weight did not correlate well with AUC_{0-12h}.

Table 5 Pharmacokinetic parameters of linezolid

	Linezolid daily dosage				
	400 mg (n=5)	600 mg (n=41)	900 mg (n=7)	1,200 mg (n=5)	p-value
C_{min} (mg/L)	3.7 (1.9)	2.8 (1.8)	3.0 (1.5)	4.6 (5.0)	0.405
C_{max} (mg/L)	11.0 (3.8)	9.0 (3.1)	9.3 (2.5)	10.6 (3.1)	0.666
AUC_{0-12h} (mg*h/L)	83.3 (33.9)	65.9 (30.8)	71.3 (19.7)	89.2 (49.3)	0.472
AUC/MIC ratio	499 (375)	357 (252)*	432 (135)	707 (499)	0.193

Data presented as mean (standard deviation). Pharmacokinetic parameters of patients that concomittantly received P-gp modulators are excluded.* n=37 due to 4 missing MICs.
P-values calculated using Independent Samples Kruskal-Wallis Test displaying whether the distribution of pharmacokinetic parameters is the same across all linezolid dosage groups, *i.e.* 400 mg, 600 mg, 900 mg, and 1,200 mg. A non-parametric test was used because of the small number of samples.

DISCUSSION

This is the first study to investigate linezolid toxicity in relation to pharmacokinetic linezolid exposure. The efficacy of treatment regimens containing linezolid in this cohort was very good, with a large percentage of MDR-TB patients that was considered cured. The median times observed to microscopy and culture conversion (both 47 days) were similar to those of a recent meta-analysis; in that analysis, microscopy converted to negative after median 43.5 days, and culture after 61 days (10). The proportion of adverse events in the retrospective cohort was lower than previously reported in a large meta-analysis (10). In our cohort, anemia occurred in 16%; peripheral neuropathy in 19%; and optical neuritis in 2% of all patients; anemia was seen in 38%, peripheral neuropathy in 47%, optic neuritis in 13% of patients in the meta-analysis (10). Comparison of dose related effects is difficult due to the multitude of the included studies in the meta-analysis, the non-fixed dose in our retrospective study and small overlap of data (10).

In Haren, 75% of the included patients received erythropoietin in a dose of 2,000 IUs twice a week. Erythropoietine was given in an attempt to prevent anemia which could otherwise lead to cessation of an effective drug in MDR-TB patients. Analysis of the Hb levels revealed no significant differences between patients that did or did not receive preventive erythropoietine. As no evidence for any benefit emerged from this analysis, we would now argue against routine use of erythropoietine in patients receiving linezolid.

Peripheral neuropathy was observed in 19% of the patients. The cumulative linezolid dosage and the number of days patients were exposed to linezolid were observed to be statistically significantly higher in patients who experienced peripheral neuropathy. A possible explanation that cumulative linezolid dosage and days of exposure to linezolid, in contrast to linezolid C_{min} or AUC, are correlated to peripheral neuropathy might be that even the lower linezolid C_{min} and AUCs are above mitochondrial toxicity threshold (28).

International collaboration in MDR/XDR-TB research is critically important to merge data and reach meaningful cohort sizes to explore important clinical questions. The toxicity data we found, *e.g.* for anemia and leucopenia in patients with higher C_{min} could only be detected by merging data of our two centers. The fact that in the same analysis, C_{min} did not predict optical neuritis and peripheral neuropathy could be due to the limited sample size, but moment of pharmacokinetic sampling in relation to the occurrence of adverse events amongst others.

Several methodological study limitations can be identified, particularly the limited sample size and the retrospective epidemiological nature of our study, and missing data. Unfortunately, 43 patients had to be excluded, because of lack of TDM data or due to recent admission to the hospital. Despite the fact that the inclusion and exclusion process has been transparently documented, this could have led to a selection bias. Furthermore, pharmacokinetic linezolid data were cross-sectionally computed at one or few moments in time. During the course of treatment, linezolid dosages or clinical parameters might have changed. The differences between the two study settings is another relevant limitation. Data were collected at two hospitals by two different researchers. We cannot exclude general differences in standard of care, treatment, and monitoring. The impact of observed differences in treatment regimens is expected to be limited since most differences are seen in drugs with a disputable place in therapy (6). Furthermore, differences were inherent to the individual character of treatment regimens.

Despite the retrospective nature of this study and the above mentioned limitations, our study supports the increasingly important position of linezolid in MDR-TB treatment regimens. Toxicity is more frequent with higher doses, *i.e.* 600 mg twice daily (15). However, with reduced dosages, adverse events appear manageable and infrequent. Our findings in this retrospective study justify confirmation in a prospective study.

One of the most important questions that remains to be answered is what pharmacokinetic / pharmacodynamic target should be aimed for in the treatment of MDR-TB? There are suggestive *in vitro* data for *M. tuberculosis* to support an AUC/MIC ratio of 100 which is adequate for gram-positive organisms (18). In addition, in vivo data showed promising results of linezolid added to other anti tubercular agents (29). Our study showed a weak association between AUC/MIC ratio and days to sputum smear-microscopy or to culture conversion in the presence of multi drug regimen limited by its retrospective nature. However, target AUC/MIC might be obtained in *in vitro* pharmacodynamic infection models that simulate human pharmacokinetics, such as hollow-fiber models. Next, prospective PK/PD studies should be designed to validate these targets in MDR-TB patients. Perhaps, linezolid dried blood spot and a limited sampling strategy could facilitate TDM in such studies (30). Being able to taper the dose to the lowest effective linezolid dosage with minimal toxicity based on TDM together with affordable, generic linezolid in safe and effective treatment regimen containing new agents, such as PA-824, bedaquiline, and delamanid, could help in the management of tuberculosis worldwide.

In conclusion, our retrospective study suggests that the treatment regimens containing linezolid were effective and well tolerated, with high sputum microscopy and culture conversion rates and low frequency of adverse events. Interestingly, peripheral neuropathy seems to be mediated by cumulative dose and days of exposure to linezolid. There was no relevant correlation between pharmacokinetic/pharmacodynamic parameters and patients characteristics.

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